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LETTER TO THE EDITOR

Reply: Interaction between Brassinosteroids and Gibberellins: Synthesis or Signaling? In Arabidopsis, Both! OPEN

Brassinosteroids (BRs) are plant hormones with versatile roles. Among other functions, the BRs control cell elongation, division, and differentiation events (Gudesblat and Russinova, 2011), fulfill roles in adaptive growth processes (Wang et al., 2012; Fridman and Savaldi-Goldstein, 2013), and participate in abiotic and biotic stress responses (Kagale et al., 2007; De Bruyne et al., 2014; Eremina et al., 2016). Their many roles throughout the plant life cycle are enabled by a central signaling module, which controls the expression of thousands of target genes (Sun et al., 2010; Yu et al., 2011). This module has been heavily studied in the last two decades, thereby considerably improving our understanding, which has been recently summarized in a number of excellent reviews (Guo et al., 2013; Wang et al., 2014; Singh and Savaldi-Goldstein, 2015). Here, we summarize our integrated model of BR-GA crosstalk (Unterholzner et al., 2015) and explain why we consider BRs as "master regulators" of GA biosynthesis.

BR AND GA BIOSYNTHESIS AND SIGNALING ARE INTERTWINED IN ARABIDOPSIS

When the first BR deficient mutants of *Arabidopsis thaliana* were isolated (Clouse et al., 1996; Li et al., 1996; Szekeres et al., 1996), it became evident that BRs may act synergistically with GAs in aspects of growth control (Steber and McCourt, 2001). In Arabidopsis and other species, BR mutants phenotypically resemble GA-deficient plants in impaired germination (Unterholzner et al., 2015), hypocotyl elongation, dwarfism, darker green leaves, late flowering, and reduced fertility (Clouse, 2011). Numerous studies have shown that BRs and GAs interact at the signaling level (Bai et al., 2012; Gallego-Bartolomé et al., 2012; Li et al., 2012; Bernardo-García et al., 2014). GAs signal through the degradation of the DELLA transcriptional repressors (Schwechheimer, 2012; Davière and Achard, 2013), which is initiated following the perception of bioactive GA by the GA receptor GID1 (Griffiths et al., 2006; Willige et al., 2007). There is good evidence that DELLAs interact with the BR-related transcription factors BES1 and BZR1 and repress their activities on target promoters (Bai et al., 2012; Gallego-Bartolomé et al., 2012; Li et al., 2012).

Because hypocotyl elongation of BR mutant seedlings could not be restored with externally applied GA in Arabidopsis (Li et al., 1996; Szekeres et al., 1996; Bai et al., 2012; Gallego-Bartolomé et al., 2012), it was postulated that BRs do not regulate GA biosynthesis but that the crosstalk is restricted to the signaling level. Therefore, it was surprising that when determining the GA contents in Arabidopsis BR mutants, levels of the major bioactive GA, GA_4, were clearly reduced (Unterholzner et al., 2015). This finding prompted us to readdress a possible role of BRs in GA biosynthesis in this species.

Since an impact of BRs on GA biosynthesis had been excluded in Arabidopsis based solely on experiments performed on seedlings, we expanded the experiments to additional growth stages. This confirmed the earlier results: In the seedling stage, the hypocotyls of the severe BR mutants *bri1-1* and *cpd* responded only weakly to GA when grown on media containing Murashige and Skoog (MS) salts. However, on media without MS salts, the mutants were fully responsive to GA. Moreover, GA almost completely restored the growth defects of these mutants during germination and control of flowering time (Unterholzner et al., 2015). Thus, contrary to what is implied by Ross and Quittenden (2016), externally applied GA can be effective on severe BR mutants, but this depends on the developmental stage and the physiological conditions, suggesting that additional factors influence the process.

When we addressed the molecular mode of BR activity in GA biosynthesis, we found that the expression of multiple genes encoding enzymes of the GA20ox and GA3ox families, in particular *GA20ox1* and *GA3ox1*, were significantly reduced in BR mutants (Unterholzner et al., 2015), confirming earlier reports (Bouquin et al., 2001; Gallego-Bartolomé et al., 2012). Because *GA20ox1* expression was enhanced in the dominant *bes1-D* and *bzr1-1D* mutants, we studied the role of BES1 in the process. Using in vitro and in vivo DNA binding studies, we showed that BES1 can directly bind to a previously unknown binding motif present in the promoters of several GA biosynthesis genes in a BR-induced manner (Unterholzner et al., 2015). GA measurements in *ASKθ-oe*, a line in which BR signaling is constitutively repressed (Rozhon et al., 2010), provided evidence that BRs are required for the synthesis of several GAs, namely, GA_{15}, GA_{24}, GA_9, and GA_4. Whereas the bioactive GA GA_4 was reduced 5-fold, GA_9, its biosynthetic precursor and the product of GA20ox activity, was reduced 16-fold in *ASKθ-oe* compared with that in the wild type (Unterholzner et al., 2015). Therefore, there is evidence that BRs are master regulators of GA biosynthesis in Arabidopsis because they directly regulate not only one but multiple steps of this central metabolic pathway.

With these results, we proposed an expanded model for BR-GA activity in the growth control of Arabidopsis (Unterholzner et al., 2015, Figure 8), where BRs induce GA production to release DELLA-repressive effects on BES1/BZR1 in a feed-forward mode, promoting joined BR-GA responses. In this model, BR-GA cooperation at the signaling level is of equal importance as the function of BRs in GA synthesis because without the former the later is ineffective. In our view, there is no need to envision two separate models; rather, we expanded upon the existing model in which signaling clearly plays a key role.

In Arabidopsis, the function of BRs in GA biosynthetic gene expression is complemented

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by a role of GAs in BR biosynthetic gene expression (Stewart Lilley et al., 2013). Therefore, BRs and GAs act in a highly interlinked manner in Arabidopsis, impacting each other's synthesis and cooperating in signaling. That said, it is clear that both hormones have additional roles that do not depend on each other (see Tong and Chu, 2016), and in Arabidopsis, this is supported by the observation that when *GA20ox* expression is reconstituted in the weak *bri1-301* allele, some growth defects are restored whereas others remain (Unterholzner et al., 2015).

DEVELOPMENTAL STAGE AND ENVIRONMENTAL CONTEXT INFLUENCE BR AND GA ACTIVITIES

GA biosynthesis is highly regulated by exogenous stimuli (Stavang et al., 2005, 2010; Weller et al., 2009), which must be taken into account when GA-related phenotypes are studied. For example, in BR mutants, a 4°C treatment fully recovered germination defects (Unterholzner et al., 2015), indicating that cold may act independently of BRs to induce GA biosynthesis during germination.

In addition, GA homeostasis is closely monitored and adjusted via GA signaling. In particular, the DELLAs contribute to the feedback control of GA production because transcript levels of *GA20ox* and *GA3ox* genes are low in mutants lacking DELLAs but are strongly elevated in plants containing stabilized DELLA forms (Dill et al., 2001; Zentella et al., 2007; Weston et al., 2008). Interestingly, this feedback control appears to be inactive in BR mutants because despite low levels of bioactive GA, *GA20ox* and *GA3ox* expression is not induced (Unterholzner et al., 2015). Therefore, it is possible that BRs participate in the feedback control of GA biosynthesis, and this could be achieved by a regulatory role of DELLAs in BES1/BZR1 activities also in GA biosynthesis. Because DELLAs promote the expression of the BES1 targets *GA20ox1* and *GA3ox1* (Dill et al., 2001; Zentella et al., 2007; Weston et al., 2008; Gallego-Bartolomé et al., 2012) but repress BES1 activity on the promoters of other GA signaling-responsive genes (Bai et al., 2012; Gallego-Bartolomé et al., 2012; Li et al., 2012; Zentella et al., 2016), it will be interesting to determine how such potential dual activities of BES1/BZR1+DELLA-containing protein complexes may be realized. It is probable that, depending on spatiotemporal requirements, both developmental context and environmental setting govern the assembly of these complexes.

SPECIES- AND TISSUE-SPECIFIC DIFFERENCES

Matters are further complicated by species-specific differences that may occur in the functions of BRs during GA biosynthesis. In tomato (*Solanum lycopersicum*), GA application rescues aspects of BR mutant phenotypes (Bishop et al., 1999; Nomura et al., 2005; Martí et al., 2006). Micro-Tom, a mutant defective in *DWF4* expression, responds to the application of GA_3 with increased growth (Martí et al., 2006), and phenotypes of the severe BR mutant d^x, which is deficient in *CYP85A1* expression (Bishop et al., 1999; Nomura et al., 2005), could be partially restored with GA_3. In particular, mean stem height, number of internodes per plant, and mean internode length were significantly increased. In contrast, abnormal leaf morphology was not released in d^x (Nadhzimov et al., 1988). In this respect, it is interesting that, although other phenotypes were rescued, the leaf morphology in the Arabidopsis *bri1-301* mutant could not be restored by reestablishing *GA20ox* expression in the *BRI1* expression domains (Unterholzner et al., 2015). When GAs were measured, d^x showed significantly increased GA_{20} levels; GA_1 was not quantified (Nadhzimov et al., 1988).

Recently, Li et al. (2016) showed that $d^{\wedge im}$, a weak *cyp85a1* mutant of tomato, had increased GA_{20} levels, whereas GA_1 levels were not altered. Increased GA_{20} levels were also observed in the pea (*Pisum sativum*) BR biosynthetic mutant *lkb*, whereas the level of bioactive GA_1 was slightly reduced to slightly increased, depending on the tissue investigated (Lawrence et al., 1992; Jager et al., 2005). Interestingly, the application of brassinolide (BL) significantly reduced the elevated level of GA_{20} in *lkb* and *lk*; thus, the authors concluded that "the reduction of GA_{20} levels in BR-deficient mutants after the application of BL indicates a clear effect of BRs on the GA biosynthesis pathway" (Jager et al., 2005). However, how this effect is mediated remains to be investigated.

For rice, there is evidence that BR deficiency impairs GA production because GA_1 was decreased in the BR-deficient mutants *d11*, *GSK2oe*, and *dlt*, whereas it was increased in the BR overaccumulating line *m107*. When GA intermediates were determined in the BR overaccumulating line, products of GA20ox activities were decreased, whereas GA_1, the product of GA3ox activities, was increased. This correlated with corresponding changes in the GA biosynthetic gene expression in the mutants (Tong et al., 2014). Therefore, there is evidence that in rice, similar to Arabidopsis, BRs control GA biosynthesis through the regulation of GA biosynthetic gene expression, albeit by different means.

These past studies used different tissues for measurements, and the growth conditions strongly varied, ranging from soil growth to hydroponic culture systems, from 10 to 18 h daylength, and from 21 to 30°C daytime temperature. Light levels and spectra remained mostly undefined. Furthermore, the genetic backgrounds used are not easily comparable because they differ in the degree of BR or BR signaling deficiency. In future studies that measure GAs or determine other GA responses in BR mutants, it will be important that the same developmental stages, tissues, and mutants of comparable severity are used. Moreover, it will be critical that the same growth conditions are applied to be able to make statements regarding similarities or differences between plant species.

It is evident that our knowledge of the interplay of BRs and GAs is just beginning to form. At this stage, we know too little regarding the molecular modes of BR and GA activities during gene regulation, be it on promoters of GA biosynthesis genes or targets further downstream during signaling, to postulate dogmatic models. Moreover, linear insulated pathways, as proposed by Ross and Quittenden (2016), do not reflect the complex events of hormonal signaling cascades, which are highly interconnected with other signaling modules. Developmental phase and species-specific regulatory modes yet to be revealed will further expand our knowledge of BR and GA interplay during growth control and other processes, such as stress responses, which are yet to be studied.

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REFERENCES

Bai, M.Y., Shang, J.X., Oh, E., Fan, M., Bai, Y., Zentella, R., Sun, T.P., and Wang, Z.Y. (2012). Brassinosteroid, gibberellin and phytochrome impinge on a common transcription module in Arabidopsis. Nat. Cell Biol. **14:** 810–817.

Bernardo-García, S., de Lucas, M., Martínez, C., Espinosa-Ruiz, A., Davière, J.M., and Prat, S. (2014). BR-dependent phosphorylation modulates PIF4 transcriptional activity and shapes diurnal hypocotyl growth. Genes Dev. **28:** 1681–1694.

Bishop, G.J., Nomura, T., Yokota, T., Harrison, K., Noguchi, T., Fujioka, S., Takatsuto, S., Jones, J.D., and Kamiya, Y. (1999). The tomato DWARF enzyme catalyses C-6 oxidation in brassinosteroid biosynthesis. Proc. Natl. Acad. Sci. USA **96:** 1761–1766.

Bouquin, T., Meier, C., Foster, R., Nielsen, M.E., and Mundy, J. (2001). Control of specific gene expression by gibberellin and brassinosteroid. Plant Physiol. **127:** 450–458.

Clouse, S.D. (2011). Brassinosteroids. The Arabidopsis Book **9:** e0151, doi/10.1199/tab.0151.

Clouse, S.D., Langford, M., and McMorris, T.C. (1996). A brassinosteroid-insensitive mutant in Arabidopsis thaliana exhibits multiple defects in growth and development. Plant Physiol. **111:** 671–678.

Davière, J.M., and Achard, P. (2013). Gibberellin signaling in plants. Development **140:** 1147–1151.

De Bruyne, L., Höfte, M., and De Vleesschauwer, D. (2014). Connecting growth and defense: the emerging roles of brassinosteroids and gibberellins in plant innate immunity. Mol. Plant **7:** 943–959.

Dill, A., Jung, H.S., and Sun, T.P. (2001). The DELLA motif is essential for gibberellin-induced degradation of RGA. Proc. Natl. Acad. Sci. USA **98:** 14162–14167.

Eremina, M., Rozhon, W., and Poppenberger, B. (2016). Hormonal control of cold stress responses in plants. Cell. Mol. Life Sci. **73:** 797–810.

Fridman, Y., and Savaldi-Goldstein, S. (2013). Brassinosteroids in growth control: how, when and where. Plant Sci. **209:** 24–31.

Gallego-Bartolomé, J., Minguet, E.G., Grau-Enguix, F., Abbas, M., Locascio, A., Thomas, S.G., Alabadí, D., and Blázquez, M.A. (2012). Molecular mechanism for the interaction between gibberellin and brassinosteroid signaling pathways in Arabidopsis. Proc. Natl. Acad. Sci. USA **109:** 13446–13451.

Griffiths, J., Murase, K., Rieu, I., Zentella, R., Zhang, Z.L., Powers, S.J., Gong, F., Phillips, A.L., Hedden, P., Sun, T.P., and Thomas, S.G. (2006). Genetic characterization and functional analysis of the GID1 gibberellin receptors in Arabidopsis. Plant Cell **18:** 3399–3414.

Gudesblat, G.E., and Russinova, E. (2011). Plants grow on brassinosteroids. Curr. Opin. Plant Biol. **14:** 530–537.

Guo, H., Li, L., Aluru, M., Aluru, S., and Yin, Y. (2013). Mechanisms and networks for brassinosteroid regulated gene expression. Curr. Opin. Plant Biol. **16:** 545–553.

Jager, C.E., Symons, G.M., Ross, J.J., Smith, J.J., and Reid, J.B. (2005). The brassinosteroid growth response in pea is not mediated by changes in gibberellin content. Planta **221:** 141–148.

Kagale, S., Divi, U.K., Krochko, J.E., Keller, W.A., and Krishna, P. (2007). Brassinosteroid confers tolerance in *Arabidopsis thaliana* and *Brassica napus* to a range of abiotic stresses. Planta **225:** 353–364.

Lawrence, N.L., Ross, J.J., Mander, L.N., and Reid, J.B. (1992). Internode length in Pisum. Mutants lk, lka and lkb do not accumulate gibberellins. J. Plant Growth Regul. **11:** 35–37.

Li, J., Nagpal, P., Vitart, V., McMorris, T.C., and Chory, J. (1996). A role for brassinosteroids in light-dependent development of Arabidopsis. Science **272:** 398–401.

Li, Q.F., Wang, C., Jiang, L., Li, S., Sun, S.S., and He, J.X. (2012). An interaction between BZR1 and DELLAs mediates direct signaling crosstalk between brassinosteroids and gibberellins in Arabidopsis. Sci. Signal. **5:** ra72.

Li, X.J., et al. (2016). DWARF overexpression induces alteration in phytohormone homeostasis, development, architecture and carotenoid accumulation in tomato. Plant Biotechnol. J. **14:** 1021–1033.

Martí, E., Gisbert, C., Bishop, G.J., Dixon, M.S., and García-Martínez, J.L. (2006). Genetic and physiological characterization of tomato cv. Micro-Tom. J. Exp. Bot. **57:** 2037–2047.

Nadhzimov, U.K., Jupe, S.C., Jones, M.G., and Scott, I.M. (1988). Growth and gibberellin relations of the extreme dwarf d^x tomato mutant. Physiol. Plant. **73:** 252–256.

Nomura, T., Kushiro, T., Yokota, T., Kamiya, Y., Bishop, G.J., and Yamaguchi, S. (2005). The last reaction producing brassinolide is catalyzed by cytochrome P-450s, CYP85A3 in tomato and CYP85A2 in Arabidopsis. J. Biol. Chem. **280:** 17873–17879.

Ross, J.J., and Quittenden, L.J. (2016). Interactions between brassinosteroids and gibberellins: Synthesis or Signaling? Plant Cell **28:** 829–832.

Rozhon, W., Mayerhofer, J., Petutschnig, E., Fujioka, S., and Jonak, C. (2010). ASKtheta, a group-III Arabidopsis GSK3, functions in the brassinosteroid signalling pathway. Plant J. **62:** 215–223.

Schwechheimer, C. (2012). Gibberellin signaling in plants - the extended version. Front. Plant Sci. **2:** 107.

Singh, A.P., and Savaldi-Goldstein, S. (2015). Growth control: brassinosteroid activity gets context. J. Exp. Bot. **66:** 1123–1132.

Stavang, J.A., Lindgård, B., Erntsen, A., Lid, S.E., Moe, R., and Olsen, J.E. (2005). Thermoperiodic stem elongation involves transcriptional regulation of gibberellin deactivation in pea. Plant Physiol. **138:** 2344–2353.

Stavang, J.A., Pettersen, R.I., Wendell, M., Solhaug, K.A., Junttila, O., Moe, R., and Olsen, J.E. (2010). Thermoperiodic growth control by gibberellin does not involve changes in photosynthetic or respiratory capacities in pea. J. Exp. Bot. **61:** 1015–1029.

Steber, C.M., and McCourt, P. (2001). A role for brassinosteroids in germination in Arabidopsis. Plant Physiol. **125:** 763–769.

Stewart Lilley, J.L., Gan, Y., Graham, I.A., and Nemhauser, J.L. (2013). The effects of DELLAs on growth change with developmental stage and brassinosteroid levels. Plant J. **76:** 165–173.

Sun, Y., et al. (2010). Integration of brassinosteroid signal transduction with the transcription

network for plant growth regulation in Arabidopsis. Dev. Cell **19:** 765–777.

Szekeres, M., Németh, K., Koncz-Kálmán, Z., Mathur, J., Kauschmann, A., Altmann, T., Rédei, G.P., Nagy, F., Schell, J., and Koncz, C. (1996). Brassinosteroids rescue the deficiency of CYP90, a cytochrome P450, controlling cell elongation and de-etiolation in Arabidopsis. Cell **85:** 171–182.

Tong, H., and Chu, C. (2016). Reply: Brassinosteroid regulates gibberellin synthesis to promote cell elongation in rice: critical comments on Ross and Quittenden's letter. Plant Cell **28:** 833–835.

Tong, H., Xiao, Y., Liu, D., Gao, S., Liu, L., Yin, Y., Jin, Y., Qian, Q., and Chu, C. (2014). Brassinosteroid regulates cell elongation by modulating gibberellin metabolism in rice. Plant Cell **26:** 4376–4393.

Unterholzner, S.J., Rozhon, W., Papacek, M., Ciomas, J., Lange, T., Kugler, K.G., Mayer, K.F., Sieberer, T., and Poppenberger, B. (2015). Brassinosteroids are master regulators of gibberellin biosynthesis in Arabidopsis. Plant Cell **27:** 2261–2272.

Wang, W., Bai, M.Y., and Wang, Z.Y. (2014). The brassinosteroid signaling network-a paradigm of signal integration. Curr. Opin. Plant Biol. **21:** 147–153.

Wang, Z.Y., Bai, M.Y., Oh, E., and Zhu, J.Y. (2012). Brassinosteroid signaling network and regulation of photomorphogenesis. Annu. Rev. Genet. **46:** 701–724.

Weller, J.L., Hecht, V., Vander Schoor, J.K., Davidson, S.E., and Ross, J.J. (2009). Light regulation of gibberellin biosynthesis in pea is mediated through the COP1/HY5 pathway. Plant Cell **21:** 800–813.

Weston, D.E., Elliott, R.C., Lester, D.R., Rameau, C., Reid, J.B., Murfet, I.C., and Ross, J.J. (2008). The Pea DELLA proteins LA and CRY are important regulators of gibberellin synthesis and root growth. Plant Physiol. **147:** 199–205.

Willige, B.C., Ghosh, S., Nill, C., Zourelidou, M., Dohmann, E.M., Maier, A., and Schwechheimer, C. (2007). The DELLA domain of GA INSENSITIVE mediates the interaction with the GA INSENSITIVE DWARF1A gibberellin receptor of Arabidopsis. Plant Cell **19:** 1209–1220.

Yu, X., Li, L., Zola, J., Aluru, M., Ye, H., Foudree, A., Guo, H., Anderson, S., Aluru, S., Liu, P., Rodermel, S., and Yin, Y. (2011). A brassinosteroid transcriptional network revealed by genome-wide identification of BESI target genes in *Arabidopsis thaliana*. Plant J. **65:** 634–646.

Zentella, R., Zhang, Z.L., Park, M., Thomas, S.G., Endo, A., Murase, K., Fleet, C.M., Jikumaru, Y., Nambara, E., Kamiya, Y., and Sun, T.P. (2007). Global analysis of della direct targets in early gibberellin signaling in Arabidopsis. Plant Cell **19:** 3037–3057.

Zentella, R., et al. (2016). O-GlcNAcylation of master growth repressor DELLA by SECRET AGENT modulates multiple signaling pathways in Arabidopsis. Genes Dev. **30:** 164–176.